533 Rec'd PCT/PTO 09 AUG 2001

FORM PTO-1390 (Modified) (REV 11-98) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER: **PF 91 PCT SEQ / dln**

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR): **09/913107**

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/FROO/OO394	**17 FEB 2000 (17.02.00)**	**17 FEB 1999 (17.02.99)**

TITLE OF INVENTION

USE OF AN ENTEROBACTERIUM OmpA PROTEIN ASSOCIATED WITH THE ELAGITILTV PEPTIDE, FOR TREATING MELANOMES

APPLICANT(S) FOR DO/EO/US

Toufic RENNO, Pedro ROMERO, Isabelle MICONNET, Jean-Charles CAROTTINI, and Jean-Yves BONNEFOY

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☐ This is an express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. 371 (c) (2))
   a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☒ has been transmitted by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☒ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ☒ A copy of the International Search Report (PCT/ISA/210).
8. ☐ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☐ have not been made and will not be made.
9. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
10. ☒ An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)).
11. ☒ A copy of the International Preliminary Examination Report (PCT/IPEA/409).
12. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).

Items 13 to 20 below concern document(s) or information included:

13. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
14. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
15. ☒ A **FIRST** preliminary amendment.
16. ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
17. ☐ A substitute specification.
18. ☐ A change of power of attorney and/or address letter.
19. ☒ Certificate of Mailing by Express Mail
20. ☒ Other items or information:

PTO 1449 listing references of International Search Report
Abstract of the Disclosure in U.S. format
Sequence Listing - Paper and Diskette
Statement under 37 CFR 1.821(f)

PCTUS1/REV03

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER
09/913107	PGT/FROO/OO394	PF 91 PCT SEQ / dln

21. The following fees are submitted:.

BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :

		CALCULATIONS PTO USE ONLY
☐	Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2) paid to USPTO and International Search Report not prepared by the EPO or JPO **$1,000.00**	
☒	International preliminary examination fee (37 CFR 1.482) not paid to USPTO but Internation Search Report prepared by the EPO or JPO **$860.00**	
☐	International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO **$710.00**	
☐	International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4) **$690.00**	
☐	International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4) **$100.00**	
	ENTER APPROPRIATE BASIC FEE AMOUNT =	**$860.00**
	Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (e)).	**$0.00**

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	
Total claims	22 - 20 =	2	x $18.00	**$36.00**
Independent claims	2 - 3 =	0	x $80.00	**$0.00**
Multiple Dependent Claims **(check if applicable)**.			☐	**$0.00**

TOTAL OF ABOVE CALCULATIONS =		**$896.00**
Reduction of 1/2 for filing by small entity, if applicable. Verified Small Entity Statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28) **(check if applicable).**	☐	**$0.00**
SUBTOTAL =		**$896.00**
Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (f)). +		**$0.00**
TOTAL NATIONAL FEE =		**$896.00**
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) **(check if applicable).**	☐	**$0.00**
TOTAL FEES ENCLOSED =		**$896.00**
	Amount to be: refunded	$
	charged	$

☒ A check in the amount of **896** to cover the above fees is enclosed.

☐ Please charge my Deposit Account No. in the amount of to cover the above fees.
A duplicate copy of this sheet is enclosed.

☒ The Commissioner is hereby authorized to charge any fees which may be required, or credit any overpayment to Deposit Account No. **8-3220** A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

G. Patrick Sage
THE FIRM OF HUESCHEN AND SAGE
500 Columbia Plaza
350 East Michigan Ave.
Kalamazoo, MI 49007

G. Patrick Sage
SIGNATURE

G. PATRICK SAGE
NAME

37,710
REGISTRATION NUMBER

August 2, 2001
DATE

* * * * *

Applicant : Toufic Renno, Pedro Romero, Isabelle Miconnet, Jean-Charles Carottini, and Jean-Yves Bonnefoy

Title : USE OF AN ENTEROBACTERIUM OmpA PROTEIN ASSOCIATED WITH THE ELAGITILTV PEPTIDE, FOR TREATING MELANOMES

* * * * *

Honorable Commissioner of Patents and Trademarks
Washington, D.C. 20231

PRELIMINARY AMENDMENT

Sir:

A soon as a Serial Number and Filing Date have been accorded the above-identified national phase application, kindly amend as follows:

IN THE SPECIFICATION: Kindly insert the enclosed Sequence Listing after the last page of the description and before the claims.

IN THE CLAIMS: Kindly cancel all of the claims, 1 through 24, and replace by Claims 25 through 48 attached.

IN THE ABSTRACT: Attached please find an Abstract of the Disclosure in U.S. format.

R E M A R K S

The present application is a national phase filing of PCT/FR00/00394 of February 17, 2000.

Applicants have cancelled all of the originally-filed Claims, 1 through 24. New Claims, 25 through 48, have been added to better encompass the full scope and breadth of the invention notwithstanding Applicants belief that the Claims would have been allowable as originally filed. Accordingly, Applicants assert that no Claims have been narrowed within the meaning of *Festo*.

A U.S. format Abstract is provided.

A PTO 1449 listing all of the references cited in the International Search Report is provided.

Entry of the new Claims and Abstract and early and favorable action on the merits of this application are respectfully solicited.

Respectfully submitted,

THE FIRM OF HUESCHEN AND SAGE

By: G. PATRICK SAGE

G. PATRICK SAGE

Dated: August 9, 2001
Customer No.: 25,666
500 Columbia Plaza
350 East Michigan Ave.
Kalamazoo, MI 49007
(616) 382-0030

Enclosure: Postal Card Receipt
Sequence Listing - Paper copy
Sequence Listing - diskette copy
Abstract of the Disclosure
Claims 25 through 48
PTO 1449

CLAIMS

25. The use of an enterobacterium OmpA protein, or of a fragment thereof, associated with the peptide of sequence ELAGIGILYV SEQ ID No. 3, for preparing a pharmaceutical composition useful in generating a cytotoxic T response directed against melanoma cells.

26. The use of an enteroacterium OmpA protein, or of a fragment thereof, associated with the peptide of SEQ ID No. 3, as claimed in claim 25, for preparing a pharmaceutical composition useful in treating or preventing malignant melanomas.

27. The use of claim 25, wherein said enterobacterium OmpA protein, or a fragment thereof, is obtained using a method of extraction from a culture of said enterobacterium.

28. The use of claim 25, wherein said enterobacterium OmpA protein, or a fragment thereof, is obtained via the recombinant route,

29. The use of claim 25, wherein said enterobacterium is *Klebsiella pneumoniae.*

30. The use of claim 29, wherein the amino acid sequence of said OmpA protein, or a fragment thereof, is selected form the group consisting of :

a) the amino acid sequence of SEQ ID No. 2;

b) the amino acid sequence of a sequence having at least 80% homology with SEQ ID No. 2; and

c) the amino acid sequence of a fragment of at least 5 amino acids of a sequence as defined in a).

31. The use of claim 25, wherein said peptide of SEQ ID No. 3 is coupled to or mixed with said OmpA protein or a fragment thereof.

32. The use of claim 30, wherein said peptide of SEQ ID No. 3 is coupled, by covalent attachment, with said OmpA protein or a fragment thereof.

33. The use of claim 32, wherein the coupling by covalent attachment is produced by chemical synthesis.

34. The use of claim 33, wherein one or more attachment elements is (are) introduced into said OmpA protein, or a fragment thereof, and/or into said peptide of SEQ ID No. 3, in order to facilitate the chemical coupling.

35. The use of claim 34, wherein said attachment element introduced is an amino acid.

36. The use of claim 32, wherein the hybrid protein resulting from the coupling between said peptide of SEQ ID No. 3 and said OmpA protein, or a fragment thereof, is obtained by genetic recombination.

37. The use of claim 36, wherein the pharmaceutical composition comprises a nucleic acid construct encoding said hybrid protein.

38. The use of claim 37, wherein said nucleic acid construct is contained in a vector, or in a transformed host cell capable of expressing said hybrid protein.

39. The use of claim 25, for preparing a pharmaceutical composition which can be administered by the subcutaneous or intradermal route.

40. The use of claim 25, wherein said pharmaceutical composition is vehicled in a form which makes it possible to improve its stability and/or its immunogenicity.

41. A pharmaceutical composition of claim 25.

42. The pharmaceutical composition of claim 41, wherein the protein is selected from the group consisting of:

1) *Klebsiella pneumoniae* OmpA protein of SEQ ID No. 2;

2) a protein, the sequence of which has at least 80% homology with the SEQ ID No. 2; and

3) a fragment of at least 5 amino acids of said OmpA protein of SEQ ID No. 2;

the protein being associated, by mixing or by coupling, with the peptide of SEQ ID No. 3.

43. A pharmaceutical composition, wherein the protein is selected from the group consisting of

1) a nucleic acid construct containing a nucleic acid encoding the *Klebsiella pneumoniae* OmpA protein of SEQ ID No. 2;

2) a protein, the sequence of which has at least 80% homology with SEQ ID No. 2; and

3) a fragment of at least 5 amino acids of said OmpA protein of sequence SEQ ID No. 2;

and a nucleic acid encoding the peptide of sequence SEQ ID No. 3.

44. The composition of claim 41, wherein said pharmaceutical composition is vehicled in a form which makes it possible to improve its stability and/or its immunogenicity.

45. The composition of claim 44, wherein said vehicle is a liposome, or a viral vector, or a transformed host cell capable of expressing said OmpA protein, or a fragment thereof, and said peptide of SEQ ID No. 3.

46. The composition of claim 41, wherein said composition is contained in a pharmaceutically acceptable medium.

47, The composition of claim 41, wherein said composition also contains a detergent.

48. The composition of claim 41, without any other adjuvant for inducing a CTL response.

USE OF AN ENTEROBACTERIUM OmpA PROTEIN ASSOCIATED WITH THE ELAGIGILTV PEPTIDE, FOR TREATING MELANOMAS

The invention relates to the use of an enterobacterium, in particular *Klebsiella pneumoniae*, OmpA membrane protein, associated with an antigen or a hapten, for preparing a pharmaceutical composition intended to generate or increase a cytotoxic T response directed against an infectious agent or a tumor cell. The invention comprises the use of these compounds for preventing and treating infection or cancer, in particular cancers associated with a tumor antigen, such as melanomas, and also for pharmaceutical compositions comprising some of these compounds.

Vaccination is an effective means of preventing or reducing viral or bacterial infections. The success of vaccination campaigns in these domains has made it possible to extend the vaccine concept, until now used in the domain of infectology, to the domains of cancer and of autoimmune diseases. Vaccinal antigens administered alone to the host are often not immunogenic enough to induce an immune response and must, therefore, be associated with an adjuvant or coupled to a carrier protein in order to induce (or increase) the immunogenicity. Under these conditions, only an immune response of the humoral type can be induced. Now, in the context of antiviral therapy, the generation of cytotoxic T lymphocytes (CTLs) capable of recognizing and destroying the virus is of great importance (Bachmann et al., 1994, Eur. J. Immunol., 24, 2228-2236; Borrow P., 1997, J. Virol. Hepat., 4, 16-24), as attested by many studies showing, *in vivo*, the protective role of responses directed against viral epitopes (Arvin AM, 1992, J. Inf. Dis., 166, S 35-S41; Koszinowski et al., 1987 Immunol. Lett., 16, 185-192).

The importance of CTL responses has also been greatly documented in antitumor responses, in particular those

directed against melanoma cells (review in Rivoltini et al., 1998, Crit. Rev. Immunol. 18, 55-63). The CTL epitope(s) (peptide sequences which interact with class I molecules and are presented to CD8+ T lymphocytes) have been defined for several antigens.

However, the difficulty lies in generating CTLs *in vivo*, due to the weak immunogenicity of these peptides (Melief, 1992, Adv. Cancer Res., 58, 143-175; Nandaz and Sercaz, 1995, Cell, 82, 13-17).

Research is consequently directed toward identifying novel adjuvants, or of an antigen delivery system, making it possible to induce CTLs. Due to their effectiveness in presenting antigens and in stimulating the immune system, dendritic cells, for example, have been used to generate antiviral CTL responses (Ludewig B et al., 1998, J. Virol., 72, 3812-3818; Brossard P. et al., 1997, J. Immunol., 158, 3270-3276) or anticancer CTL responses (Nestle F.O. et al., 1998, Nat. Med., 4, 328-332). The approaches have consisted in loading the dendritic cells *ex vivo*, with the antigen of interest (peptides or cell lysate) and reimplanting these cells into the patient. Other approaches consist in transfecting, *ex vivo*, the dendritic cells with the gene encoding the antigen of interest and in reinjecting these transfected cells (Gilboa E. et al., 1998, Cancer Immunol. Immunother., 46, 82-87). These approaches have been used successfully in mice and recently in humans (Hsu F.J. et al., 1996, Nat. Med., 2, 52-58), but nevertheless remain complex since the cells must be treated *ex vivo* (transformation of the cells or internalization of the antigens) and transplanted into the host organism. Similarly, the use of viral-type particles (Layton G.T. et al., 1993, J. Immunol., 151, 1097-1107) or of incomplete Freund's adjuvant (IFA) (Valmori et al., Eur. J. Immunol., 1994, 24, 1458-1462) makes it possible to generate CTL responses. However, antiviral

corresponding to CTL epitopes and in the presence of such an adjuvant may lead to a state of specific tolerance, which may, in certain cases, produce the opposite effect to that desired, i.e. a decrease in the immune response (Toes et al., Proc. Nat. Acad. Sci. USA, 1996, 93, 7855-7860).

Thus, there exists, today, a great need for a compound which, when associated with a molecule, in particular an antigen or hapten, is capable of generating CTLs directed against said molecule. Such a compound could, in particular, be used for preparing a vaccinal composition intended to induce immune protection of the antiviral, antibacterial, antifungal, antiparasitic or antitumor CTL type.

Surprisingly, it has been demonstrated that an outer membrane protein of a gram-negative bacterium, in particular an enterobacterium OmpA protein such as the *Klebsiella pneumoniae* P40 protein (protein described in WO 95/27787 and WO 96/14415), has the property of eliciting a CTL response against a molecule which is covalently or noncovalently associated with it, preferably without having to add another adjuvant.

Thus, the present invention relates to the use of an enterobacterium OmpA protein, of a fragment thereof or of a nucleic acid sequence encoding said OmpA protein or a fragment thereof, for preparing a pharmaceutical composition intended to generate or increase a cytotoxic T response against an infectious agent or a tumor cell, *in vitro* or *in vivo*, preferably *in vivo*, and also for preparing a pharmaceutical composition intended to generate or increase said cytotoxic T response.

In the present invention, the term "protein" is intended to denote both peptides or polypeptides and

the term "OmpA" (for "outer membrane protein") is intended to denote outer membrane proteins of the A type.

The expression "fragment of an OmpA protein" is intended to denote, in particular, any fragment of amino acid sequence included in the amino acid sequence of the OmpA protein which, when it is associated with an antigen or hapten specific for an infectious agent or for a tumor cell, is capable of generating or increasing a cytotoxic T response directed against said infectious agent or said tumor cell, said fragment of the OmpA protein comprising at least 5 amino acids, preferably at least 10 amino acids or more preferably at least 15 amino acids.

The expression "antigen or hapten specific for an infectious agent or for a tumor cell" is intended to denote, in particular, any compound expressed by an infectious agent, such as a virus, a bacterium, a yeast, a fungus or a parasite, or by a tumor cell, or a structural analog thereof, which, alone or in association with an adjuvant of immunity, is capable of inducing an immune response specific for said infectious agent or for said tumor cell.

In the present description, the expression "analog of an antigen or hapten" is intended to denote, in particular, a compound having structural similarity with said antigen or hapten, capable of inducing an immunological response directed against said antigen or hapten in an organism immunized beforehand with said similar compound.

A subject of the invention is also the use as claimed in the invention, characterized in that said pharmaceutical composition also comprises, associated with said enterobacterium OmpA protein, an antigen or a

hapten specific for said infectious agent or for said tumor cell.

Preferably, the invention comprises the use as claimed in the invention, characterized in that said infectious agent is a viral particle, a bacterium, a yeast, a fungus or a parasite.

In a particular embodiment, the invention comprises the use of an enterobacterium OmpA protein, or of a fragment thereof, as claimed in the invention, characterized in that said enterobacterium OmpA protein, or a fragment thereof, is obtained using a method of extraction from a culture of said enterobacterium.

The methods for extracting bacterial membrane proteins are known to those skilled in the art and will not be developed in the present description. Mention may, for example, be made, but without being limited thereto, of the extraction method described by Haeuw J.H. et al. (Eur. J. Biochem, 255, 446-454, 1998).

In another preferred embodiment, the invention also comprises the use of an enterobacterium OmpA protein, or of a fragment thereof, as claimed in the invention, characterized in that said enterobacterium OmpA protein, or a fragment thereof, is obtained via the recombinant route.

The methods for preparing the recombinant proteins are, today, well known to those skilled in the art and will not be developed in the present description; reference may however be made to the method described in the examples. Among the cells which may be used for producing these recombinant proteins, mention should, of course, be made of bacterial cells (Olins P.O. and Lee S.C., 1993, Recent advances in heterologous gene expression in E. coli. Curr. Op. Biotechnology

4:520-525), and also yeast cells (Buckholz R.G., 1993, Yeast Systems for the Expression of Heterologous Gene Products. Curr. Op. Biotechnology 4:538-542), as well as animal cells, in particular mammalian cell cultures (Edwards C.P. and Aruffo A., 1993, Current applications of COS cell based transient expression systems. Curr. Op. Biotechnology 4:558-563), and also insect cells in which methods may be used which implement, for example, baculoviruses (Luckow V.A., 1993, Baculovirus systems for the expression of human gene products. Curr. Op. Biotechnology 4:564-572).

Entirely preferably, the use as claimed in the invention is characterized in that the said enterobacterium is *Klebsiella pneumoniae*.

In particular, the invention relates to the use as claimed in the invention, characterized in that the amino acid sequence of said *Klebsiella pneumoniae* OmpA protein, or a fragment thereof, comprises:

a) the amino acid sequence of sequence SEQ ID No. 2;
b) the amino acid sequence of a sequence having at least 80%, preferably 90% and 95% homology, after optimal alignment, with the sequence SEQ ID No. 2; or
c) the amino acid sequence of a fragment of at least 5 amino acids of a sequence as defined in a).

The expression "nucleic acid or amino acid sequence with at least 80% homology, after optimal alignment, with a given nucleic acid or amino acid sequence" is intended to denote a sequence which, after optimal alignment with said given sequence, comprises a percentage identity of at least 80% with said given sequence.

For the purposes of the present invention, the term "percentage identity" between two nucleic acid or amino

acid sequences is intended to denote the percentage of nucleotides or of amino acid residues which are identical between the two sequences to be compared, obtained after the best alignment, this percentage being purely statistical and the differences between the two sequences being distributed randomly and over their entire length. Sequence comparisons between two nucleic acid or amino acid sequences are conventionally carried out by comparing these sequences after having aligned them optimally, said comparison being carried out by segment or by "window of comparison" in order to identify and compare local regions of sequence similarity. The optimal alignment of the sequences for comparison may be produced, other than manually, by means of the local homology algorithm of Smith and Waterman (1981) [Ad. App. Math. 2:482], by means of the local homology algorithm of Neddleman and Wunsch (1970) [J. Mol. Biol. 48:443], by means of the similarity search method of Pearson and Lipman (1988) [Proc. Natl. Acad. Sci. USA 85:2444], by means of computer software which uses these algorithms (GAP, BESTFIT, FASTA and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, WI, or with BLAST N or BLAST P comparison software).

The percentage identity between two nucleic acid or amino acid sequences is determined by comparing these two sequences which are optimally aligned by the window of comparison in which the region of the nucleic acid or amino acid sequence to be compared may comprise additions or deletions with respect to the reference sequence for optimal alignment between these two sequences. The percentage identity is calculated by determining the number of identical positions for which the nucleotide or the amino acid residue is identical between the two sequences, dividing this number of identical positions by the total number of positions in the window of comparison and multiplying the result

obtained by 100 in order to obtain the percentage identity between these two sequences.

Use may, for example, be made of the BLAST program "BLAST 2 sequences", which is available on the site http://www.ncbi.nlm.nih.gov/gorf/bl2.html, the parameters used being those given by default (in particular for the "open gap penalty" parameter: 5, and the "extension gap penalty" parameter: 2; the matrix chosen being, for example, the "BLOSUM 62" matrix provided by the program), the percentage identity between the two sequences to be compared being calculated directly by the program.

Among said sequences having at least 80% homology with the reference OmpA sequence, preference is given to the sequences of, or encoding, peptides capable of inducing CTL activity directed specifically against the antigen or hapten which is associated with it, such as the CTL activity measured using the standard techniques described in the examples hereinafter.

The invention also comprises the use as claimed in the invention, characterized in that said antigen or hapten is chosen from proteins, lipopeptides, polysaccharides, oligosaccharides, nucleic acids, lipids or any compound capable of specifically directing the CTL response against said infectious antigen or said tumor cell.

A subject of the present invention is also the use as claimed in the invention, characterized in that said antigen or hapten is coupled to or mixed with said OmpA protein or a fragment thereof.

The invention also comprises the use as claimed in the invention, characterized in that said antigen or hapten is coupled by covalent attachment, in particular by chemical coupling, with said OmpA protein or a fragment thereof.

In a particular embodiment, the use as claimed in the invention is characterized in that one or more attachment elements is(are) introduced into said OmpA protein, or a fragment thereof, and/or into said antigen or hapten, in order to facilitate the chemical coupling; preferably, said attachment element introduced is an amino acid.

As claimed in the invention, it is possible to introduce one or more attachment elements, in particular amino acids, in order to facilitate the coupling reactions between the OmpA protein, or a fragment thereof, and said antigen or hapten. The covalent coupling between the OmpA protein, or a fragment thereof, and said antigen or hapten as claimed in the invention may be carried out at the N- or C-terminal end of the OmpA protein or a fragment thereof. The difunctional reagents which enable this coupling will be determined as a function of the end of the OmpA protein, or a fragment thereof, which is chosen for carrying out the coupling, and of the nature of said antigen or hapten to be coupled.

In another particular embodiment, the use as claimed in the invention is characterized in that the coupling between said antigen or hapten and said OmpA protein, or a fragment thereof, is produced by genetic recombination, when said antigen or hapten is peptide in nature.

The conjugates derived from coupling to said OmpA protein, or a fragment thereof, may be prepared by genetic recombination. The chimeric or hybrid protein (conjugate) may be produced using recombinant DNA techniques, by inserting or adding a sequence encoding said antigen or hapten which is peptide in nature into the DNA sequence encoding said OmpA protein or a fragment thereof.

The methods for synthesizing the hybrid molecules encompass the methods used in genetic engineering for constructing hybrid polynucleotides encoding desired polypeptide sequences. Advantageously, reference may, for example, be made to the technique for obtaining genes encoding fusion proteins, described by D.V. Goeddel (Gene expression technology, Methods in Enzymology, Vol. 185, 3-187, 1990).

In another aspect, the invention relates to the use as claimed in the invention, characterized in that the pharmaceutical composition comprises a nucleic acid construct encoding said hybrid protein, or comprises a vector containing a nucleic acid construct encoding said hybrid protein or a transformed host cell containing said nucleic acid construct, which is capable of expressing said hybrid protein.

The invention also comprises the use as claimed in the invention, for preparing a pharmaceutical composition intended to eliminate infectious agents or inhibit tumor growth.

Preferably, the use as claimed in the invention relates to the preparation of a pharmaceutical composition intended to prevent or treat infectious diseases or cancers, preferably cancers associated with a tumor antigen.

Among cancers in which the tumors express an associated tumor antigen, and which may be prevented or treated with the uses as claimed in the present invention, mention may be made, in particular, but without being limited thereto, of:

- breast cancer, lung cancer, colon cancer and gastric carcinoma (Kawashima et al., 1999, Cancer Res. 59:431-5);

- mesothelioma, osteosarcoma, brain cancers (Xie et al., 1999, J. Natl. Cancer. Inst. 91:169-75);
- melanoma (Zheuten et al., 1998, Bratilsl. Lek. Listy 99:426-34);
- cystic adinoma of the pancreas (Hammel et al., 1998, Eur. J. gastroenterol. Hepatol. 10:345-8);
- colorectal cancer (Ogura et al., 1998, Anticancer Res. 18:3669-75);
- renal cell carcinoma (Jantzer et al., 1998, Cancer Res. 58:3078-86); and
- cancer of the ovary and of the cervix (Sonoda et al., 1996, Cancer. 77:1501-9).

A subject of the invention is in particular the use of an enterobacterium OmpA protein, or of a fragment thereof, as claimed in the invention, for preparing a pharmaceutical immunization composition intended to prevent or treat an infectious disease, in particular of viral, bacterial, fungal or parasitic origin, or a cancer, preferably associated with a tumor antigen, in particular melanomas.

The invention also comprises the use as claimed in the invention, characterized in that said pharmaceutical composition is vehicled in a form which makes it possible to improve its stability and/or its immunogenicity, in particular in the form of a liposome.

Preferably, the invention comprises the use as claimed in the invention, characterized in that said vehicle is a viral vector containing a nucleic acid construct encoding said OmpA protein or a fragment thereof, said antigen or hapten, or said hybrid protein, or a transformed host cell capable of expressing said OmpA protein or a fragment thereof, said antigen or hapten, or said hybrid protein.

The invention also comprises the use as claimed in the invention, characterized in that said nucleic acid construct, or the nucleic acid construct contained in said vector or said transformed host cell, comprises a nucleic acid sequence chosen from the sequence SEQ ID No. 1, a fragment thereof having at least 15 consecutive nucleotides, preferably 30 consecutive nucleotides, of the sequence SEQ ID No. 1, or a sequence having at least 80% homology, after optimal alignment, with one of said sequences.

Preferably, a subject of the present invention is the use of an enterobacterium OmpA protein, or of a fragment thereof, associated with an antigen or a hapten, for preparing a pharmaceutical composition intended to generate a cytotoxic T response directed against a tumor cell, as claimed in the present invention, characterized in that said antigen or hapten is the peptide of sequence SEQ ID No. 3: ELAGIGILTV and in that the cytotoxic T response is directed against melanoma cells.

Also preferably, a subject of the present invention is the use of an enterobacterium OmpA protein, or a fragment thereof, associated with the peptide of sequence ELAGIGILTV, as claimed in the present invention, for preparing a pharmaceutical composition intended to treat or prevent malignant melanomas.

In another aspect, a subject of the invention is a pharmaceutical composition as defined above, in particular:

- a pharmaceutical composition, characterized in that it comprises an enterobacterium OmpA protein, or a fragment thereof, associated, by mixing or by covalent coupling, with the peptide of sequence ELAGIGILTV; or
- a pharmaceutical composition, characterized in that it comprises a nucleic acid construct containing a

nucleic acid encoding an enterobacterium OmpA protein, or a fragment thereof, and a nucleic acid encoding the peptide of sequence ELAGIGILTV.

As defined above for the use as claimed in the invention, said pharmaceutical composition as claimed in the invention may, for example, comprise an enterobacterium OmpA protein, or a fragment thereof, coupled, by covalent attachment, to the peptide of sequence ELAGIGILTV by chemical synthesis, using recombinant OmpA or OmpA obtained via an extraction method, or coupled by genetic recombination.

As also defined above for the use as claimed in the invention, said pharmaceutical composition as claimed in the invention may, for example, comprise a vector comprising a nucleic acid construct containing a nucleic acid encoding an enterobacterium OmpA protein, or a fragment thereof, and/or a nucleic acid encoding the peptide of sequence ELAGIGILTV, or alternatively a transformed cell capable of expressing an enterobacterium OmpA protein, or a fragment thereof, and/or the peptide of sequence ELAGIGILTV.

A subject of the invention is also a pharmaceutical composition, characterized in that it comprises the *Klebsiella pneumoniae* OmpA protein of sequence SEQ ID No. 2, a protein, the sequence of which has at least 80% homology, after optimal alignment, with the sequence SEQ ID No. 2, or a fragment of at least 5 amino acids of said OmpA protein of sequence SEQ ID No. 2, associated, by mixing or by coupling, with the peptide of sequence ELAGIGILTV.

A subject of the present invention is also a pharmaceutical composition, characterized in that it comprises a nucleic acid construct containing a nucleic acid encoding the *Klebsiella pneumoniae* OmpA protein of sequence SEQ ID No. 2, a protein, the sequence of which

has at least 80% homology, after optimal alignment, with the sequence SEQ ID No. 2, or a fragment of at least 5 amino acids of said OmpA protein of sequence SEQ ID No. 2, and a nucleic acid encoding the peptide of sequence ELAGIGILTV.

According to the present invention, said compositions will be vehicled in a form which makes it possible to improve its stability and/or its immunogenicity, such as in the form of a liposome, or of a viral vector or of a transformed host cell capable of expressing said OmpA protein, or a fragment thereof, and said peptide of sequence ELAGIGILTV.

According to the present invention, said compositions will preferably be contained in a pharmaceutically acceptable medium.

For the purposes of the present invention, the pharmaceutically acceptable medium is the medium in which the compounds of the invention are administered, preferably a medium which can be injected into humans. It may consist of water, of an aqueous saline solution or of an aqueous solution based on dextrose and/or on glycerol.

According to the present invention, said compositions may also contain a detergent.

The compositions as claimed in the invention may also contain a detergent, and in particular any type of pharmaceutically acceptable surfactant, such as for example anionic, cationic, nonionic or amphoteric surfactants. Use is preferably made of the detergents Zwittergent 3-12 and octylglucopyrannoside, and even more preferably Zwittergent 3-14.

The invention also comprises the compositions as claimed in the invention, characterized in that they contain no other adjuvant for inducing a CTL response. Preferably, said pharmaceutical composition as claimed in the invention contains no adjuvant of immunity, besides the enterobacterium OmpA protein, or a fragment thereof, or a nucleic acid encoding the enterobacterium OmpA protein, or a fragment thereof, characteristic of the pharmaceutical compositions of the invention.

The legends to the figures and examples which follow are intended to illustrate the invention without in any way limiting the scope thereof.

Legends to the figures:

Figures 1A, 1B, 1C and 1D: Measurement of the anti-MELAN-A and anti-TRP-2 CTL activity of effector cells

After immunization with 50 µg of hELA mixed with 3 µg of rP40 (figure 1A), 50 µg of hELA mixed with 300 µg of rP40 (figure 1B), 50 µg of hELA coupled to rP40 (figure 1C) or 50 µg of the TRP-2 peptide mixed with 300 µg of rP40 (figure 1D), the draining lymph mode cells are stimulated with EL-4 A2/Kb cells (figures 1A, 1B and 1C) or EL-4 cells (figure 1D) which had been irradiated and prepulsed with 1 µM of the relevant peptide, before being evaluated for their capacity to kill target cells which may (rectangle) or may not (diamond) have been prepulsed with the relevant peptide.

The X-axes of the points of figures 1A to 1D correspond to the ratio of the effector T cells (active lymphocytes) mixed together with the target cells (EL-4 A2/Kb or EL-4).

Figures 2A, 2B, 2C and 2D: Measurement of the anti-MELAN-A CTL activity of effector cells in the presence

of the rP40 protein compared to the CTL activity obtained with standard immunization protocol.

After immunization with hELA (50 µg) alone (ELA, figure 2A), hELA mixed with 300 µg of rP40 (ELA + P40, figure 2B), hELA coupled to 300 µg of rP40 (ELA/P40, figure 2C) or hELA mixed with 50 µg of P30 peptide adjuvanted with IFA (ELA + IFA + TT, figure 2D) (IFA for Incomplete Freund's Adjuvant and TT for Tetanus Toxoid), the draining lymph node cells are stimulated in vitro for two weeks with EL-4 A2/Kb cells which have been irradiated and prepulsed with 1 µM of the relevant peptide, before being evaluated for their capacity to kill EL-4 A2/Kb target cells which may (rectangle) or may not (diamond) have been prepulsed with the hELA peptide.

Example 1: Cloning of the gene encoding the *Klebsiella pneumoniae* P40 protein

The gene encoding the P40 protein was obtained by PCR amplification using the genomic DNA of *Klebsiella pneumoniae* IP I145 (Nguyen et col., Gene, 1998). The gene fragment encoding this gene is inserted into various expression vectors under the control of various promoters, in particular that of the Trp operon. The nucleotide sequence and the peptide sequence of the P40 protein are represented by the sequences SEQ ID No. 1 and SEQ ID No. 2 hereinafter. An *E. coli* K12 producer strain was transformed with a pvaLP40 expression vector. The recombinant P40 protein (named rP40) is produced, in the form of inclusion bodies, with a considerable yield (> 10% in g of proteins/g of dry biomass).

This example is merely an illustration of the expression of the rP40 protein, this illustration possible being extended to other bacterial strains and to other expression vectors.

Example 2: Method for fermentation of rP40 fusion proteins

An Erlenmeyer flask containing 250 ml of TSB (Tryptic Soy Broth, Difco) medium containing ampicilline (100 µg/ml, Sigma) and tetracyclin (8 µg/ml, Sigma) is inoculated with the transformed *E. coli* strain described above. After overnight incubation at 37°C, 200 ml of this culture are used to seed 2 liters of culture medium in a fermenter (Biolaffite, France). In a quite conventional way, the culture medium may be composed of chemical agents supplemented with vitamins and/or yeast extracts, which are known to promote high density bacterial cell growth.

The parameters controlled during the fermentation are: pH, stirring, temperature, level of oxygenation and supply of combined sources (glycerol or glucose). In general, the pH is regulated at 7.0 and the temperature is fixed at 37°C. The growth is controlled by supplying glycerol (87%) at a constant rate (12 ml/h) in order to maintain the dissolved oxygen tension signal at 30%. When the turbidity of the culture (measured at 580 nm) reaches the value of 80 (after culturing for approximately 24 hours), the protein production is treated by adding indole acrylic acid (IAA) at the final concentration of 25 mg/l. Approximately 4 hours after induction, the cells are harvested by centrifugation. The amount of wet biomass obtained is approximately 200 g.

Example 3: Method for extracting and purifying the rP40 protein

Extracting the rP40

After centrifugation of the culture broth (4000 rpm, 10 min, 4°C), the cells are resuspended in a 25 mM Tris-HCl buffer, pH 8.5. The insoluble components, or inclusion bodies, are obtained after treatment with

lysozyme (0.5 g/liter, 1 hour at room temperature with gentle stirring). The inclusion body pellet obtained by centrifugation (15 min at 10,000 g at 4°C) is taken up in a 25 mM Tris-HCl buffer at pH 8.5 containing 5 mM $MgCl_2$ and then centrifuged (15 min at 10,000 g).

The inclusion bodies are solubilized at 37°C for 2 hours in a 25 mM Tris-HCl buffer, pH 8.5, containing 7 M urea (denaturing agent) and 10 mM of dithiothreitol (reduction of disulfide bridges). Centrifugation (15 min at 10,000 g) makes it possible to eliminate the insoluble particles.

This is then followed by resuspension in 13 volumes of 25 mM Tris-HCl buffer, pH 8.5, containing NaCl (8.76 g/l) and Zwittergent 3-14 (0.1%, w/v). The solution is left overnight at room temperature with gentle stirring in contact with the air (to promote renaturation of the protein by dilution and reoxidation of the disulfide bridges).

Purifying the rP40 protein

- Anion exchange chromatography step

After a further centrifugation, the solution is dialyzed against a 25 mM Tris-HCl buffer, pH 8.5, containing 0.1% Zwittergent 3-14 (100 volumes of buffer) overnight at 4°C.

The dialyzate is loaded on to a column containing a support of the strong anion exchanger type (Biorad Macro Prop High Q gel) equilibrated in the buffer described above, at a linear flow rate of 15 cm/h. The proteins are detected at 280 nm. The rP40 protein is eluted, with a linear flow rate of 60 cm/h, for an NaCl concentration of 0.2 M in the 25 mM Tris-HCl buffer, pH 8.5: 0.1% Zwittergent 3-14.

- Cation exchange chromatography step

The fractions containing the rP40 protein are pooled and concentrated by ultrafiltration with the aid of an Amicon cell system with stirring, used with a YM10-type Diaflo membrane (10 kDa cut-off threshold), for volumes of about 100 ml, or with the aid of a Millipore Minitan tangential flow filtration system, used with membrane plates having a 10 kDa cut-off threshold, for larger volumes. The fraction thus concentrated is dialyzed overnight at 4°C against a 20 mM citrate buffer, pH 3.0, containing 0.1% of Zwittergent 3-14.

The dialyzate is loaded on to a column containing a support of the strong cation exchanger type (Biorad Macro Prep High S gel) equilibrated in the 20 mM citrate buffer, pH 3.0, containing 0.1% of Zwittergent 3-14. The rP40 protein is eluted (rate 61 cm/h) for a 0.7 M NaCl concentration. The electrophoretic profiles show about a 95% degree of purity. The condition of the protein is monitored by SDS-PAGE. The P40 protein, extracted from the *Klebsiella pneumoniae* membrane, has a characteristic electrophoretic (migration) behavior depending on whether it is in denatured or native form. The native form (β-sheet structure) in fact has a lower molecular mass than the form which is denatured (α-helical structure) by the action of a denaturing agent, such as urea or guanidine hydrochloride, or by heating at 100°C in the presence of SDS. The rP40 protein is not properly renatured at the end of renaturation, regardless of whether the latter is carried out in the presence or absence of 0.1% (w/v) Zwittergent 3-14. On the other hand, total renaturation is obtained after dialysis against a 25 mM Tris/HCl buffer, pH 8.5, containing 0.1% (w/v) Zwittergent 3-14. However, it should be noted that this renaturation is only obtained when the dilution step and treatment at room temperature are, themselves, carried out in the

presence of Zwittergent 3-14 (negative results in the absence of detergent).

Example 4: Generation of CTLs

The antitumor CTL responses directed against melanoma cells were defined for several antigens. These antigens are included in one of three categories:

a) rejection antigen specific for melanoma, such as those of the MAGE family (review by van der Bruggen et al., Science 254:1643);
b) antigens resulting from the mutation of normal proteins. This group includes MUM-1 (Coulie et al., Proc. Natl. Acad. Sci. USA 92:7976-7980 (1995)); CDK4 (Wolfel et al., Science 296:1281-1284 (1995)) and HLA-A2 (Brandel et al., J. Exp. Med. 183:2501-2508 (1996));
c) differentiation antigens expressed by melanomas and melanocytes. This group includes tyrosinase (Wolfel et al., Eur. J. Immunol. 4:759 (1994) and Brichard et al., Eur. J. Immunol. 26:224 (1996)); gp 100 (Kang et al., J. Immunol. 155:1343 (1995), Cox et al., Science 264:716 (1994), and Kawakami et al., J. Immunol. 155:3961 (1995)); gp75 (Wang et al., J. Exp. Med. 183:1131 (1996)), and Mart-1/MelanA (see US patent 5,620,886).

Of all these antigens, Mart-1/MelanA appears to be the best candidate for use in immunotherapy, this being for several reasons. Firstly, this antigen was identified on the basis of the CTL response, in vivo, of the lymphocytes infiltrating the melanoma and not that, in vitro, of the peripheral blood cells, which would suggest greater relevance of this antigen in the natural response, in vivo, against melanoma (Kawakami et al., J. Exp. Med. 180:347 (1994)). In addition, Mart-1/MelanA is expressed on all melanomas examined, which makes it a preferred target for intervention by immunotherapy. Finally, peptides derived from

Mart-1/MelanA are capable of inducing a specific CTL response in patients with melanoma expressing the HLA-A2 histocompatibility antigen (Rivoltini et al., J. Immunol. 154:2257 (1995); Valmori et al., J. Immunol. 160:1750 (1998)).

HLA-A2 is the most common allele expressed in Caucasians. The CTL epitopes of Mart-1/MelanA have been defined for this allele. The antigenic peptide recognized by the majority of human CTL lines comprises amino acids 27-35 AAGIGILTV (Kawakami et al., J. Exp. Med. 180:347 (1994)). In addition, studies on the affinity of binding with HLA-A*0201 and recognition by CTL clones have demonstrated that the optimum peptide for these two functions is the 26-35 decapeptide EAAGIGILTV (Romero et al., J. Immunol. 159:2366 (1997)). However, it appears that these peptides are weakly immunogenic in vitro (Valmori et al., J. Immunol. 160:1750 (1998)) and in vivo (Jaeger et al., Int. J. Cancer 66:162 (1996)).

When comparing the amino acid sequence of the T epitopes of Mart-1/MelanA with the peptide motifs of A*0201 (Rammensee et al., Immunogenetics 41:178 (1995)), it appears that the 26-35 and 27-35 peptides have nondominant anchoring residues at position 2 and therefore weakly bind the HLA-A*0201 molecule (Kawakami et al., J. Immunol. 154:3961 (1995)), which might explain their weak immunogenicity. The international patent application published under the number WO 98/58951 describes an analog to the 26-35 peptide, in which the alanine at position 2 has been replaced with a leucine (sequence which will be named ELA).

The hELA peptide, used in the experiments below, is the subject of patent application WO 98/58951 which is the property of the Institut Ludwig de Recherche sur le Cancer [Ludwig Cancer Research Institute]. hELA is an analog of the 26-35 decapeptide (EAAGIGILTV) of

Melan-A/MART-1, which is a protein expressed on melanocytes and melanomas. Although the 26-35 decapeptide of Melan-A/MART-1 is capable of binding to the HLA-A0201 molecule (Romero et al., 1997, J. Immunol. 159, 2366-2374), it is weakly immunogenic in vitro and in vivo (Valmori et al., 1998, J. Immunol. 160, 1750-1758). The hELA analog was generated by substituting the second amino acid of the 26-35 decapeptide of Melan-A/MART-1 (an alanine) with a leucine. The result of this substitution, which is based on analysis of the residues required for anchoring the peptides to the HLA-A0201 molecule, is more effective recognition by the CTLs of patients with melanoma and better immunogenicity in vitro (Valmori et al., 1998, J. Immunol. 160, 1750-1758). HLA-A*0201/Kb (A2/Kb) transgenic mice of the strain C57Bl/6 x BDA/2 (Vitiello et al., 1991, J. Exp. Med., 173, 1007-1015) were used in this study to test ELA. The class I MHC molecule expressed in these mice is a chimeric molecule made from the α1 and α2 domains of the human HLA-A0201 molecule (the most common allotype found) and from the α3 domain of the murine K^b molecule.

The TRP-2 peptide of sequence SEQ ID No. 4 is an octapeptide corresponding to amino acids 181-188 (VYDFFVWL) of tyrosinase-related protein 2 (TRP-2). TRP-2 is expressed in melanocytes and melanomas. It has been demonstrated that this antigen induces CTL responses which protect against melanoma in C57BL/6 (H-$2K^b$) mice (Bloom et al., 1997, J. Exp. Med. 185, 453-459).

<u>A: Generation of anti-Melan-A and anti-TRP-2 CTLs after immunization with rP40 mixed with a peptide which is an analog to Melan-A or TRP-2</u>

Experimental protocol

A2/Kb mice received, by subcutaneous injection at the base of the tail:
- 50 µg of ELA mixed with 3 or 300 µg of rP40;
- 50 µg of ELA covalently coupled to 300 µg of rP40.

C57BL/6 mice received, by subcutaneous injection into the base of the tail:
- 50 µg of the TRP-2 peptide (181-188) mixed with 300 µg of rP40.

Generation of cytotoxic effector cells

10 days after immunization, the mice are sacrificed and the lymphocytes from the draining lymph nodes are recovered in order to be stimulated, in vitro, with the relevant peptide.

These lymphocytes (4 to 5 × 10^6) are cultured in a 24 well plate in DMEM plus 10 mM HEPES, 10% FCS and 50 µM β-2-mercaptoethanol, with 2 to 5 × 10^5 EL-4 A2/Kb cells or EL4 cells which have been irradiated (10 kRads) and prepulsed for 1 h at 37°C with 1 µM of the relevant peptide. After two weekly stimulations, the cells are assayed for their cytotoxic activity.

Measurement of cytotoxic activity

The EL-4 A2/Kb cells or EL4 cells are incubated for 1 h with ^{51}Cr in the presence or absence of the relevant peptide, washed and then coincubated with the effector cells at various ratios, in a 96-well plate in a volume of 200 µl for 4 to 6 h at 37°C. The cells are then centrifuged and the ^{51}Cr release is measured in 100 µl of supernatant. The percentage of specific lysis is calculated as follows:

% specific lysis = (experimental release - spontaneous release) / (total release - spontaneous release) X 100.

Results

As shown in figures 1A to 1D, the immunization of mice with an optimal dose of rP40 (300 µg) in a mixture with hELA (figure 1B) or TRP-2 (figure 1D) induces a strong specific CTL response. Such a response is also observed after immunization with rP40 coupled to hELA (figure 1C). On the other hand, the immunization with the peptide alone or rP40 alone (results not shown) or with the hELA peptide in a mixture with a suboptimal dose of rP40 (3 µg) does not induce any CTL activity (figure 1A). These results demonstrate that the rP40 molecule mixed with or coupled to immunogenic peptides makes it possible to induce a specific CTL response in vivo, this being without the addition of adjuvant.

B: Generation of anti-Melan-A CTLs after immunization with rP40 mixed with a peptide which is an analog to Melan-A, compared to a standard immunization protocol

Experimental protocol

A2/Kb mice received:

- 50 µl of IFA (incomplete Freud's adjuvant) by subcutaneous injection at the base of the tail, then, 3 weeks later, 50 µg of hELA in the presence of 50 µg of a helper-T p30 peptide derived from Tetanus Toxoid (TT) (Panina-Bordignon et al., Eur. J. Immunol., 1989, 19, 2237) adjuvanted with IFA. This protocol has been described for generating anti-peptide CTLs (Valmori et al., Eur. J. Immunol., 1994, 24, 1458) and is used as a positive control.
- 50 µg of hELA alone or 300 µg of rP40 mixed with or coupled to 50 µg of hELA.

Generation of cytotoxic effector cells

10 days after the final immunization, the mice are sacrificed and the lymphocytes from the draining lymph nodes are recovered in order to be stimulated, in vitro, with the relevant peptide.

These lymphocytes (4 to 5 x 10^6) are cultured in a 24-well plate in DMEM plus 10 mM HEPES, 10% FCS and 50 µM β-2-mercaptoethanol, with 2 to 5 x 10^5 EL-4 A2/Kb cells (murine cells transfected with the HLA-A* 0201/Kb gene) which have been irradiated (10 kRads) and prepulsed for 1 h at 37°C with 1 µM of the relevant peptide.

After one, two or three weekly stimulations, the cells are assayed for their cytotoxic activity.

The cytotoxic activity is measured according to the method described above.

Results

After immunization with nonadjuvanted rP40 coupled to hELA, an anti-hELA CTL activity comparable to that observed after immunization with hELA + P30/IFA is measured (cf. figures 2C and 2D). Similarly, the rP40 + hELA peptide mixture, itself also nonadjuvanted, generates CTLs in a way which is similar to that obtained with a conventional protocol for generating CTLs (cf. figures 2B and 2D).

No CTL activity was detected after immunization with the peptide alone (cf. figure 2A) or the rP40 protein alone (not shown), regardless of the day on which the effector cells were stimulated.

CLAIMS

1. The use of an enterobacterium OmpA protein, or of a fragment thereof, associated with the peptide of sequence SEQ ID No. 3 ELAGIGILTV, for preparing a pharmaceutical composition intended to generate a cytotoxic T response directed against melanoma cells.

2. The use of an enterobacterium OmpA protein, or of a fragment thereof, associated with the peptide of sequence SEQ ID No. 3, as claimed in claim 1, for preparing a pharmaceutical composition intended for treating or preventing malignant melanomas.

3. The use as claimed in claim 1 or 2, characterized in that said enterobacterium OmpA protein, or a fragment thereof, is obtained using a method of extraction from a culture of said enterobacterium.

4. The use as claimed in claim 1 or 2, characterized in that said enterobacterium OmpA protein, or a fragment thereof, is obtained via the recombinant route.

5. The use as claimed in one of claims 1 to 4, characterized in that said enterobacterium is *Klebsiella pneumoniae*.

6. The use as claimed in claim 5, characterized in that the amino acid sequence of said OmpA protein, or a fragment thereof, comprises:
a) the amino acid sequence of sequence SEQ ID No. 2;
b) the amino acid sequence of a sequence having at least 80% homology with the sequence SEQ ID No. 2; or

c) the amino acid sequence of a fragment of at least 5 amino acids of a sequence as defined in a).

7. The use as claimed in one of claims 1 to 6, characterized in that said peptide of sequence SEQ ID No. 3 is coupled to or mixed with said OmpA protein or a fragment thereof.

8. The use as claimed in claim 6, characterized in that said peptide of sequence SEQ ID No. 3 is coupled, by covalent attachment, with said OmpA protein or a fragment thereof.

9. The use as claimed in claim 8, characterized in that the coupling by covalent attachment is coupling produced by chemical synthesis.

10. The use as claimed in claim 9, characterized in that one or more attachment elements is(are) introduced into said OmpA protein, or a fragment thereof, and/or into said peptide of sequence SEQ ID No. 3, in order to facilitate the chemical coupling.

11. The use as claimed in claim 10, characterized in that said attachment element introduced is an amino acid.

12. The use as claimed in claim 8, characterized in that the hybrid protein resulting from the coupling between said peptide of sequence SEQ ID No. 3 and said OmpA protein, or a fragment thereof, is obtained by genetic recombination.

13. The use as claimed in claim 12, characterized in that the pharmaceutical composition comprises a nucleic acid construct encoding said hybrid protein.

14. The use as claimed in claim 13, characterized in that said nucleic acid construct is contained in a vector, or in a transformed host cell capable of expressing said hybrid protein.

15. The use as claimed in one of claims 1 to 14, for preparing a pharmaceutical composition which can be administered by the subcutaneous or intradermal route.

16. The use as claimed in one of claims 1 to 15, characterized in that said pharmaceutical composition is vehicled in a form which makes it possible to improve its stability and/or its immunogenicity.

17. A pharmaceutical composition as defined in any one of claims 1 to 16.

18. The pharmaceutical composition as claimed in claim 17, characterized in that it comprises the *Klebsiella pneumoniae* OmpA protein of sequence SEQ ID No. 2, a protein, the sequence of which has at least 80% homology with the sequence SEQ ID No. 2, or a fragment of at least 5 amino acids of said OmpA protein of sequence SEQ ID No. 2, associated, by mixing or by coupling, with the peptide of sequence SEQ ID No. 3.

19. A pharmaceutical composition, characterized in that it comprises a nucleic acid construct containing a nucleic acid encoding the *Klebsiella pneumoniae* OmpA protein of sequence SEQ ID No. 2, a protein, the sequence of which has at least 80% homology with sequence SEQ ID No. 2, or a fragment of at least 5 amino acids of said OmpA protein of sequence SEQ ID No. 2, and a nucleic acid encoding the peptide of sequence SEQ ID No. 3.

20. The composition as claimed in one of claims 17 to 19, characterized in that said pharmaceutical composition is vehicled in a form which makes it possible to improve its stability and/or its immunogenicity.

21. The composition as claimed in claim 20, characterized in that said vehicle is a liposome, or a viral vector or a transformed host cell capable of expressing said OmpA protein, or a fragment thereof, and said peptide of sequence SEQ ID No. 3.

22. The composition as claimed in one of claims 17 to 21, characterized in that said composition is contained in a pharmaceutically acceptable medium.

23. The composition as claimed in one of claims 17 to 22, characterized in that said composition also contains a detergent.

24. The composition as claimed in one of claims 17 to 23, without any other adjuvant for inducing a CTL response.

FIGURE 1A

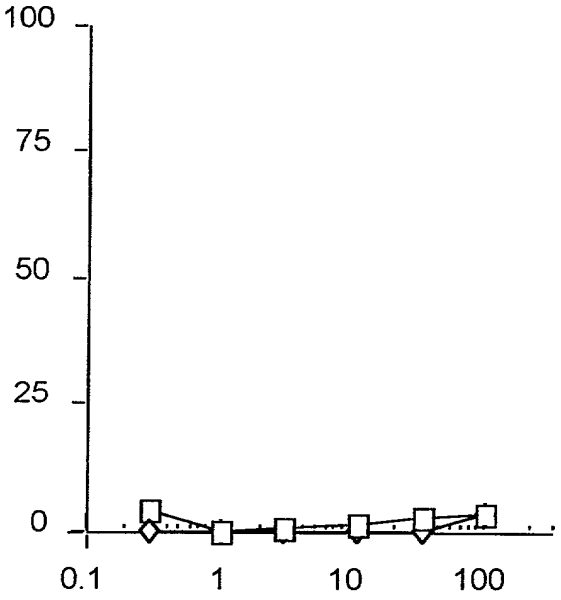


FIGURE 1B

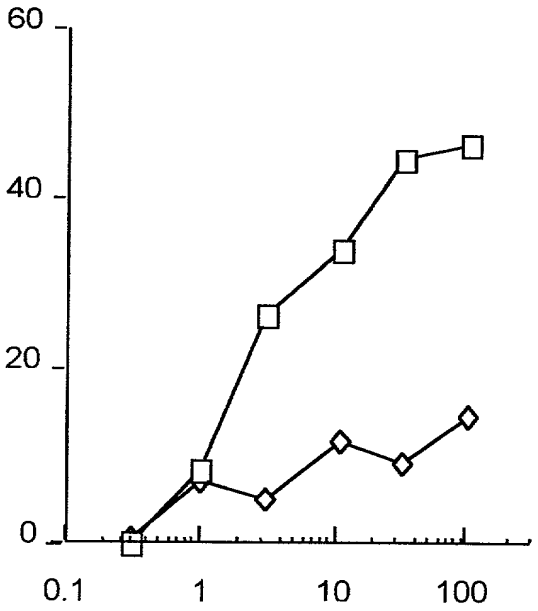


FIGURE 1C

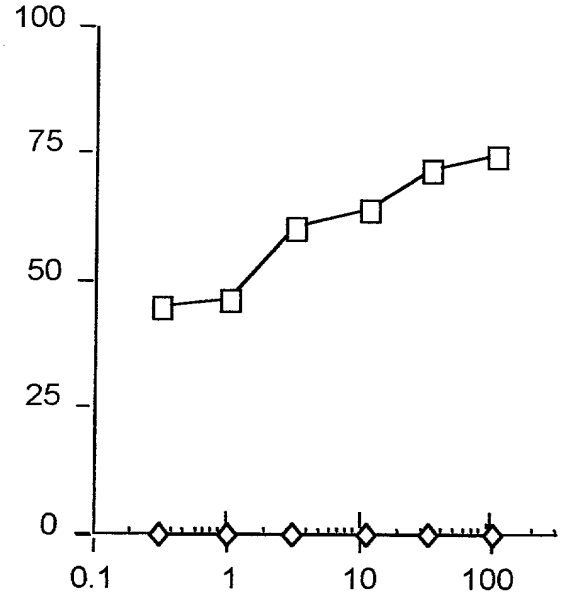
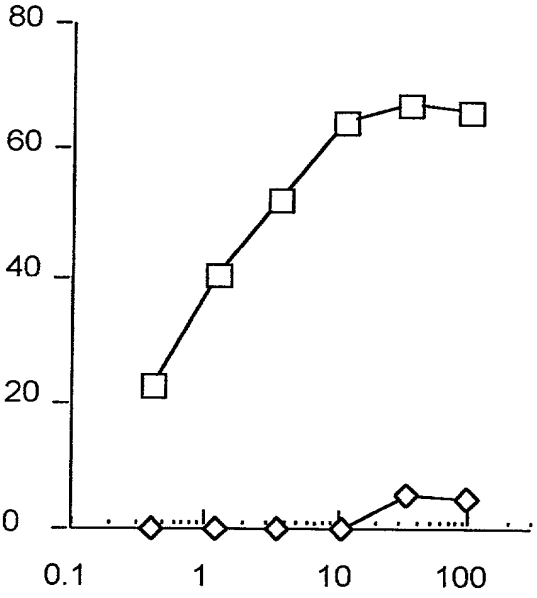


FIGURE 1D



Percentage of specific lysis

Ratio of effector cells to target cells

FIGURE 2A

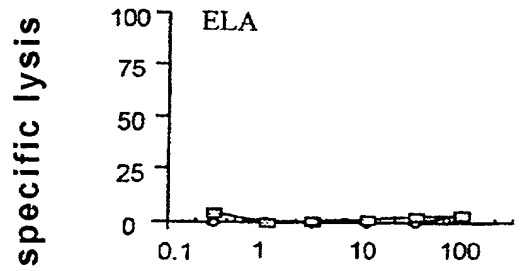


FIGURE 2B

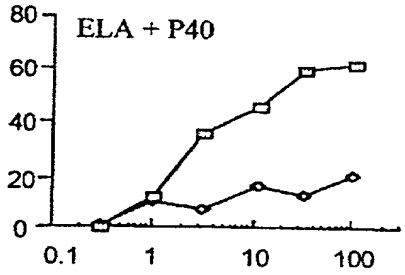


FIGURE 2C

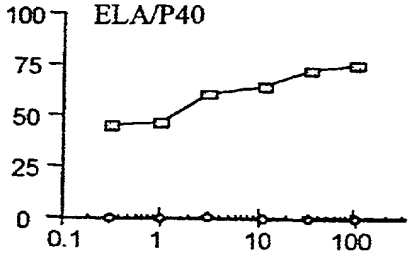
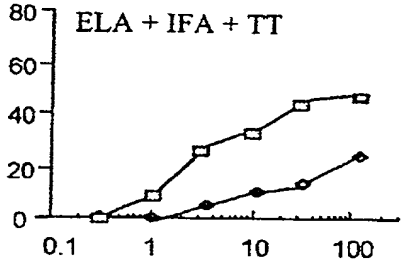


FIGURE 2D



Percentage of specific lysis

Ratio of effector cells to target cells

Please type a plus sign (+) inside this box → [+]

PTO/SB/01 (12-97)
Approved for use through 9/30/00. OMB 0651-0032
Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

DECLARATION FOR UTILITY OR DESIGN PATENT APPLICATION (37 CFR 1.63)

☒ Declaration Submitted with Initial Filing OR ☐ Declaration Submitted after Initial Filing (surcharge (37 CFR 1.16 (e)) required)

Attorney Docket Number	PF91PCTSEQ/dln
First Named Inventor	
COMPLETE IF KNOWN	
Application Number	/
Filing Date	
Group Art Unit	
Examiner Name	

As a below named inventor, I hereby declare that:

My residence, post office address, and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

USE OF AN ENTEROBACTERIUM OmpA PROTEIN ASSOCIATED WITH THE ELAGIGILTV PEPTIDE, FOR TREATING MELANOMAS

(Title of the Invention)

the specification of which

☒ is attached hereto

OR

☐ was filed on (MM/DD/YYYY) _____ as United States Application Number or PCT International Application Number _____ and was amended on (MM/DD/YYYY) _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment specifically referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR 1.56.

I hereby claim foreign priority benefits under 35 U.S.C. 119(a)-(d) or 365(b) of any foreign application(s) for patent or inventor's certificate, or 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or of any PCT international application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application Number(s)	Country	Foreign Filing Date (MM/DD/YYYY)	Priority Not Claimed	Certified Copy Attached? YES	NO
99 01917	FRANCE	17/02/99	☐	☒	☐
			☐	☐	☐
			☐	☐	☐
			☐	☐	☐

☐ Additional foreign application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto:

I hereby claim the benefit under 35 U.S.C. 119(e) of any United States provisional application(s) listed below.

Application Number(s)	Filing Date (MM/DD/YYYY)

☐ Additional provisional application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto.

Please type a plus sign (+) inside this box → [+]

PTO/SB/01 (12-97)
Approved for use through 9/30/00. OMB 0651-0032
Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

DECLARATION — Utility or Design Patent Application

I hereby claim the benefit under 35 U.S.C. 120 of any United States application(s), or 365(c) of any PCT international application designating the United States of America, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of 35 U.S.C. 112, I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application.

U.S. Parent Application or PCT Parent Number	Parent Filing Date (MM/DD/YYYY)	Parent Patent Number (If applicable)
PCT/FR00/00394	02.17.2000	

☐ Additional U.S. or PCT international application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto.

As a named inventor, I hereby appoint the following registered practitioner(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith: ☒ Customer Number 25666 → Label here 25666

OR

☐ Registered practitioner(s) name/registration number listed below

Name	Registration Number	Name	Registration Number
~~GORDON W. HUESCHEN~~	~~XX,XXX~~		
G. PATRICK SAGE	37,710		

☐ Additional registered practitioner(s) named on supplemental Registered Practitioner Information sheet PTO/SB/02C attached hereto.

Direct all correspondence to: ☒ Customer Number or Bar Code Label 25666 *OR* ☒ Correspondence address below

Name	HUESCHEN AND SAGE ~~The Firm of Gordon W. Hueschen~~ - G. PATRICK SAGE
Address	~~The "H" Building~~ 500 Columbia Plaza
Address	~~310 East Michigan Avenue~~ 350 East Michigan Ave.
City	Kalamazoo — State: MI — ZIP: 49007
Country	US — Telephone: 616-382-0030 — Fax: 616-382-2030

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Name of Sole or First Inventor: ☐ A petition has been filed for this unsigned inventor

Given Name (first and middle [if any])	Family Name or Surname
RENNO Toufic	

Inventor's Signature	Renno					Date	15.6.01
Residence: City	VIRY FRX	State		Country	FRANCE	Citizenship	LEBANON-CANADA
Post Office Address	Les Coulerins B1 - 74580 VIRY - FRANCE						
Post Office Address							
City		State		ZIP		Country	

☐ Additional inventors are being named on the ____ supplemental Additional Inventor(s) sheet(s) PTO/SB/02A attached hereto

100

Please type a plus sign (+) inside this box → [+]

PTO/SB/02A (3-97)
Approved for use through 9/30/98. OMB 0651-0032
Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

DECLARATION

ADDITIONAL INVENTOR(S)
Supplemental Sheet
Page 3 of 4

Name of Additional Joint Inventor, if any:	☐ A petition has been filed for this unsigned inventor					
Given Name (first and middle [if any])		Family Name or Surname				
ROMERO Pedro						
Inventor's Signature					**Date**	15.6.01
Residence: City	EPALINGES CH	**State**		**Country** SWITZERLAND	**Citizenship**	COLOMBIA
Post Office Address	Chemin du Polny 1 - 1066 EPALINGES - SWITZERLAND					
Post Office Address						
City		**State**	**ZIP**	**Country**		

Name of Additional Joint Inventor, if any:	☐ A petition has been filed for this unsigned inventor					
Given Name (first and middle [if any])		Family Name or Surname				
MICONNET Isabelle						
Inventor's Signature					**Date**	15.6.01
Residence: City	LAUSANNE CH	**State**		**Country** SWITZERLAND	**Citizenship**	FRANCE
Post Office Address	Chemin de Chandolin 5 - 1005 LAUSANNE - SWITZERLAND					
Post Office Address						
City		**State**	**ZIP**	**Country**		

Name of Additional Joint Inventor, if any:	☐ A petition has been filed for this unsigned inventor					
Given Name (first and middle [if any])		Family Name or Surname				
CEROTTINI Jean-Charles						
Inventor's Signature					**Date**	15.6.01
Residence: City	SAINT-SULPICE CH	**State**		**Country** SWITZERLAND	**Citizenship**	SWITZERLAND
Post Office Address	Avenue du Léman 12 - 1025 SAINT-SULPICE - SWITZERLAND					
Post Office Address						
City		**State**	**ZIP**	**Country**		

Burden Hour Statement: This form is estimated to take 0.4 hours to complete. Time will vary depending upon the needs of the individual case. Any comments on the amount of time you are required to complete this form should be sent to the Chief Information Officer, Patent and Trademark Office, Washington, DC 20231. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Assistant Commissioner for Patents, Washington, DC 20231.

Please type a plus sign (+) inside this box → [+]

PTO/SB/02A (3-97)
Approved for use through 9/30/98. OMB 0651-0032
Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

DECLARATION

ADDITIONAL INVENTOR(S)
Supplemental Sheet
Page 4 of 4

Name of Additional Joint Inventor, if any:	☐ A petition has been filed for this unsigned inventor						
Given Name (first and middle [if any])		Family Name or Surname					
BONNEFOY Jean-Yves							
Inventor's Signature						**Date**	15.6.01
Residence: City	LE SAPPEY FRX	**State**		**Country**	FRANCE	**Citizenship**	FRANCE
Post Office Address	Les Noyers - 74350 LE SAPPEY - FRANCE						
Post Office Address							
City		**State**		**ZIP**		**Country**	

Name of Additional Joint Inventor, if any:	☐ A petition has been filed for this unsigned inventor						
Given Name (first and middle [if any])		Family Name or Surname					
Inventor's Signature						**Date**	
Residence: City		**State**		**Country**		**Citizenship**	
Post Office Address							
Post Office Address							
City		**State**		**ZIP**		**Country**	

Name of Additional Joint Inventor, if any:	☐ A petition has been filed for this unsigned inventor						
Given Name (first and middle [if any])		Family Name or Surname					
Inventor's Signature						**Date**	
Residence: City		**State**		**Country**		**Citizenship**	
Post Office Address							
Post Office Address							
City		**State**		**ZIP**		**Country**	

Burden Hour Statement: This form is estimated to take 0.4 hours to complete. Time will vary depending upon the needs of the individual case. Any comments on the amount of time you are required to complete this form should be sent to the Chief Information Officer, Patent and Trademark Office, Washington, DC 20231. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Assistant Commissioner for Patents, Washington, DC 20231.

SEQUENCE LISTING

<110> PIERRE FABRE MEDICAMENT

<120> USE OF AN ENTEROBACTERIUM OmpA PROTEIN ASSOCIATED WITH THE ELAGIGILTV PEPTIDE, FOR TREATING MELANOMAS.

<130> D18441

<150> FR 99 01917
<151> 1999-02-17

<160> 4

<170> PatentIn Ver. 2.1

<210> 1
<211> 1035
<212> ADN
<213> Klebsiella pneumoniae

<220>
<221> exon
<222> (1)..(1032)

<220>
<221> intron
<222> (1033)..(1035)

<220>
<221> CDS
<222> (1)..(1032)

<400> 1

```
atg aaa gca att ttc gta ctg aat gcg gct ccg aaa gat aac acc tgg   48
Met Lys Ala Ile Phe Val Leu Asn Ala Ala Pro Lys Asp Asn Thr Trp
  1               5                  10                  15

tat gca ggt ggt aaa ctg ggt tgg tcc cag tat cac gac acc ggt ttc   96
Tyr Ala Gly Gly Lys Leu Gly Trp Ser Gln Tyr His Asp Thr Gly Phe
            20                  25                  30

tac ggt aac ggt ttc cag aac aac aac ggt ccg acc cgt aac gat cag   144
Tyr Gly Asn Gly Phe Gln Asn Asn Asn Gly Pro Thr Arg Asn Asp Gln
        35                  40                  45

ctt ggt gct ggt gcg ttc ggt ggt tac cag gtt aac ccg tac ctc ggt   192
Leu Gly Ala Gly Ala Phe Gly Gly Tyr Gln Val Asn Pro Tyr Leu Gly
    50                  55                  60

ttc gaa atg ggt tat gac tgg ctg ggc cgt atg gca tat aaa ggc agc   240
Phe Glu Met Gly Tyr Asp Trp Leu Gly Arg Met Ala Tyr Lys Gly Ser
65                  70                  75                  80

gtt gac aac ggt gct ttc aaa gct cag ggc gtt cag ctg acc gct aaa   288
Val Asp Asn Gly Ala Phe Lys Ala Gln Gly Val Gln Leu Thr Ala Lys
                85                  90                  95

ctg ggt tac ccg atc act gac gat ctg gac atc tac acc cgt ctg ggc   336
Leu Gly Tyr Pro Ile Thr Asp Asp Leu Asp Ile Tyr Thr Arg Leu Gly
```

```
                100                 105                 110

ggc atg gtt tgg cgc gct gac tcc aaa ggc aac tac gct tct acc ggc   384
Gly Met Val Trp Arg Ala Asp Ser Lys Gly Asn Tyr Ala Ser Thr Gly
        115                 120                 125

gtt tcc cgt agc gaa cac gac act ggc gtt tcc cca gta ttt gct ggc   432
Val Ser Arg Ser Glu His Asp Thr Gly Val Ser Pro Val Phe Ala Gly
    130                 135                 140

ggc gta gag tgg gct gtt act cgt gac atc gct acc cgt ctg gaa tac   480
Gly Val Glu Trp Ala Val Thr Arg Asp Ile Ala Thr Arg Leu Glu Tyr
145                 150                 155                 160

cag tgg gtt aac aac atc ggc gac gcg ggc act gtg ggt acc cgt cct   528
Gln Trp Val Asn Asn Ile Gly Asp Ala Gly Thr Val Gly Thr Arg Pro
                165                 170                 175

gat aac ggc atg ctg agc ctg ggc gtt tcc tac cgc ttc ggt cag gaa   576
Asp Asn Gly Met Leu Ser Leu Gly Val Ser Tyr Arg Phe Gly Gln Glu
            180                 185                 190

gat gct gca ccg gtt gtt gct ccg gct ccg gct ccg gct ccg gaa gtg   624
Asp Ala Ala Pro Val Val Ala Pro Ala Pro Ala Pro Ala Pro Glu Val
        195                 200                 205

gct acc aag cac ttc acc ctg aag tct gac gtt ctg ttc aac ttc aac   672
Ala Thr Lys His Phe Thr Leu Lys Ser Asp Val Leu Phe Asn Phe Asn
    210                 215                 220

aaa gct acc ctg aaa ccg gaa ggt cag cag gct ctg gat cag ctg tac   720
Lys Ala Thr Leu Lys Pro Glu Gly Gln Gln Ala Leu Asp Gln Leu Tyr
225                 230                 235                 240

act cag ctg agc aac atg gat ccg aaa gac ggt tcc gct gtt gtt ctg   768
Thr Gln Leu Ser Asn Met Asp Pro Lys Asp Gly Ser Ala Val Val Leu
                245                 250                 255

ggc tac acc gac cgc atc ggt tcc gaa gct tac aac cag cag ctg tct   816
Gly Tyr Thr Asp Arg Ile Gly Ser Glu Ala Tyr Asn Gln Gln Leu Ser
            260                 265                 270

gag aaa cgt gct cag tcc gtt gtt gac tac ctg gtt gct aaa ggc atc   864
Glu Lys Arg Ala Gln Ser Val Val Asp Tyr Leu Val Ala Lys Gly Ile
        275                 280                 285

ccg gct ggc aaa atc tcc gct cgc ggc atg ggt gaa tcc aac ccg gtt   912
Pro Ala Gly Lys Ile Ser Ala Arg Gly Met Gly Glu Ser Asn Pro Val
    290                 295                 300

act ggc aac acc tgt gac aac gtg aaa gct cgc gct gcc ctg atc gat   960
Thr Gly Asn Thr Cys Asp Asn Val Lys Ala Arg Ala Ala Leu Ile Asp
305                 310                 315                 320

tgc ctg gct ccg gat cgt cgt gta gag atc gaa gtt aaa ggc tac aaa   1008
Cys Leu Ala Pro Asp Arg Arg Val Glu Ile Glu Val Lys Gly Tyr Lys
                325                 330                 335

gaa gtt gta act cag ccg gcg ggt taa                              1035
Glu Val Val Thr Gln Pro Ala Gly
            340
```

<210> 2
<211> 344
<212> PRT
<213> Klebsiella pneumoniae

<400> 2

```
Met Lys Ala Ile Phe Val Leu Asn Ala Ala Pro Lys Asp Asn Thr Trp
  1               5                  10                  15

Tyr Ala Gly Gly Lys Leu Gly Trp Ser Gln Tyr His Asp Thr Gly Phe
             20                  25                  30

Tyr Gly Asn Gly Phe Gln Asn Asn Asn Gly Pro Thr Arg Asn Asp Gln
         35                  40                  45

Leu Gly Ala Gly Ala Phe Gly Gly Tyr Gln Val Asn Pro Tyr Leu Gly
     50                  55                  60

Phe Glu Met Gly Tyr Asp Trp Leu Gly Arg Met Ala Tyr Lys Gly Ser
 65                  70                  75                  80

Val Asp Asn Gly Ala Phe Lys Ala Gln Gly Val Gln Leu Thr Ala Lys
                 85                  90                  95

Leu Gly Tyr Pro Ile Thr Asp Asp Leu Asp Ile Tyr Thr Arg Leu Gly
            100                 105                 110

Gly Met Val Trp Arg Ala Asp Ser Lys Gly Asn Tyr Ala Ser Thr Gly
        115                 120                 125

Val Ser Arg Ser Glu His Asp Thr Gly Val Ser Pro Val Phe Ala Gly
    130                 135                 140

Gly Val Glu Trp Ala Val Thr Arg Asp Ile Ala Thr Arg Leu Glu Tyr
145                 150                 155                 160

Gln Trp Val Asn Asn Ile Gly Asp Ala Gly Thr Val Gly Thr Arg Pro
                165                 170                 175

Asp Asn Gly Met Leu Ser Leu Gly Val Ser Tyr Arg Phe Gly Gln Glu
            180                 185                 190

Asp Ala Ala Pro Val Val Ala Pro Ala Pro Ala Pro Ala Pro Glu Val
        195                 200                 205

Ala Thr Lys His Phe Thr Leu Lys Ser Asp Val Leu Phe Asn Phe Asn
    210                 215                 220

Lys Ala Thr Leu Lys Pro Glu Gly Gln Gln Ala Leu Asp Gln Leu Tyr
225                 230                 235                 240

Thr Gln Leu Ser Asn Met Asp Pro Lys Asp Gly Ser Ala Val Val Leu
                245                 250                 255

Gly Tyr Thr Asp Arg Ile Gly Ser Glu Ala Tyr Asn Gln Gln Leu Ser
            260                 265                 270

Glu Lys Arg Ala Gln Ser Val Val Asp Tyr Leu Val Ala Lys Gly Ile
        275                 280                 285
```

```
Pro Ala Gly Lys Ile Ser Ala Arg Gly Met Gly Glu Ser Asn Pro Val
    290                 295                 300

Thr Gly Asn Thr Cys Asp Asn Val Lys Ala Arg Ala Ala Leu Ile Asp
305                 310                 315                 320

Cys Leu Ala Pro Asp Arg Arg Val Glu Ile Glu Val Lys Gly Tyr Lys
                325                 330                 335

Glu Val Val Thr Gln Pro Ala Gly
            340
```

<210> 3
<211> 10
<212> PRT
<213> Homo sapiens

<220>
<223> Peptide derived from the Mart-1/MelanA antigen expressed by melanoma cells.

<400> 3
Glu Leu Ala Gly Ile Gly Ile Leu Thr Val
1   5   10

<210> 4
<211> 8
<212> PRT
<213> Homo sapiens

<220>
<223> Derivative of tyrosine-related protein 2 (TRP-2).

<400> 4
Val Tyr Asp Phe Phe Val Trp Leu
1   5